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STUDIES ON THE COLORIMETRIC DETERMINATION OF PHOSPHORUS: ACID TOLERANCE AND OPTIMUM TIME FOR COLOUR DEVELOPMENT

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Many investigators have emphasized the need of carefully controlling the acidity in some of the colorimetric methods of determining phosphorus. The useful range of acidity is one in which the reduction of molybdenum is maximum in the presence of phosphorus and minimum when no phosphorus is added. This range of acidity is spoken of by Gomori (6) as acid tolerance, and he defines it as "the permissible range of final acid concentration within which the method gives uniform readings at the optimum time suggested by the diviser of the method."

Berenblum and Chain (2) investigated the effect of acidity in the procedures outlined by Fiske and Subbarow (3) and Kuttner and Cohen (8). The latter method, in which stannous chloride is used, is very sensitive. However, it has a narrow range of acid tolerance and loses in safety what it gains in sensitivity. On the other hand, they found that the method of Fiske and Subbarow, with amino-naphthol-sulphonic acid as the reducing agent, allows a wider range of acidity and concentration of reagents, but it is less sensitive.

Gomori (6) confirmed the findings of Berenblum and Chain (2) regarding the acid tolerance of the methods of Kuttner and Cohen and Fiske and Subbarow. With hydroquinone, used in the Benedict-Theis method (1) Gomori found an excellent acid tolerance, but he pointed out the undesirable feature of boiling in this method. He also investigated the applicability of methyl-*p*-aminophenol sulphate (elon) as the reducing agent in the Fiske-Subbarow method. The intensity of colour was practically the same as with amino-naphthol-sulphonic acid. An excellent acid tolerance was found and the colour was stable for a long period of time. Gomori proposed the elon method as offering definite advantages.

Wood and Mellon (10) have presented an excellent review on the molybdenum blue reaction in which they outlined the permissible range of acidity for some of the methods.

The method devised by King (7) has been used for the determination of phosphorus in biological material in connection with the nitric-perchloric acid wet-ashing method of Gerritz (5). In view of the differences which have been found regarding the acid tolerance in other methods, it was considered advisable to investigate this factor in King's procedure in which perchloric acid is used for adjustment of the final acidity. The excellent results obtained by Gomori with elon prompted also an investigation of the applicability of this reducing agent in King's method.

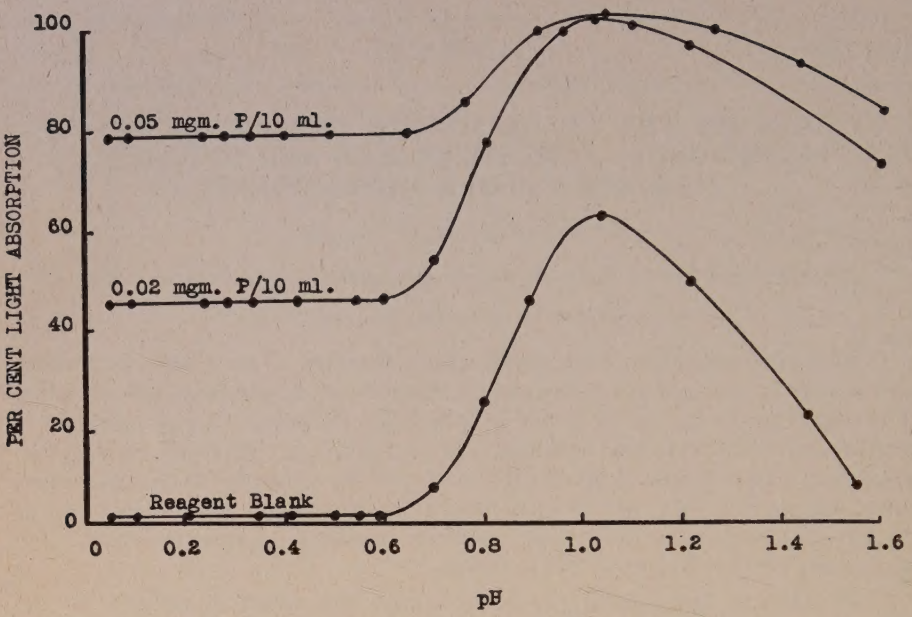

FIGURE 1. Effect of acidity on the development of the molybdenum blue colour by 1-amino-2-naphthol-4-sulphonic acid in the presence of inorganic phosphate and in the reagent blank.

EXPERIMENTAL PROCEDURE

Many of the previous investigators have discussed acidity in terms of normality and amount of acid added to the solution in which the colour is developed. The following considerations suggested the determination of the pH of the final solution rather than expressing this factor in terms of normality and amount of acid added: (1) In Gerritz wet-ash method, oxidizing acids are used to destroy the organic matter. The amount of acids left in the digest depends on the initial amount employed and the quantity that was used up for oxidation of the organic matter. This varies with the kind and amount of organic material in the sample. (2) The size of aliquot taken from the acid digest depends on the phosphorus content of the material being analysed. These factors influence to a varying degree the acidity of the final molybdenum blue solution.

In this study a standard solution of K_2HPO_4 in distilled water was used. One ml. of this solution contained 0.1 mg. of phosphorus. Suitable dilutions and aliquots were used to give the desired concentrations of phosphorus in 10 ml. volumetric flasks. To these were added varying amounts of 60 per cent perchloric acid and the solutions diluted to approximately 5 ml. with distilled water. One ml. of 5 per cent ammonium molybdate per flask was added and the contents mixed thoroughly. Then 0.5 ml. of the reducing agent was added, the solutions made up to volume, thoroughly mixed and the colour development determined at the desired time.

All colorimetric measurements were made with a Sheard-Sandford-Cenco Photelometer using colour filters to give a maximum transmission in the range 700 to 800 mu. Fontaine (4) used $SnCl_2$ and found the maximum absorption by the molybdenum blue to be at 820 mu. Lowry and Lopez

TABLE 1.—DEVELOPMENT OF MOLYBDENUM BLUE COLOUR BY 1-AMINO-2-NAPHTHOL-4-SULPHONIC ACID IN THE PRESENCE OF VARYING AMOUNTS OF INORGANIC PHOSPHATE AT pH 0.5

Mgm. of P in 10 ml.	Photometric reading time in minutes								
	2	5	10	15	20	30	40	50	60
0.01	78.8	73.4	72.8	72.4	72.0	71.0	70.0	69.0	68.2
0.04	38.0	31.0	30.0	29.0	28.6	27.6	26.2	25.0	24.0
0.07	23.4	16.0	14.0	13.0	12.6	11.6	10.5	9.6	8.0

(9) used amino-naphthol-sulphonic acid and employed a wave length of 700 mu. They stated that any wave length between 650 and 950 is satisfactory.

It was found under the conditions of the present experiments that absorption by the molybdenum blue colour produced by amino-naphthol-sulphonic acid was greatest at 800 mu as measured with a Coleman Universal Spectrophotometer, this being the upper limit of wavelength of the instrument. The absorption at 800 mu was only about 2.5 per cent greater than at 700 mu and about 5.5 per cent greater than at 660 mu.

Distilled water was employed in the blank cell for setting the photoelectric colorimeter at 100 per cent transmission. The pH determinations were made on the final molybdenum blue solution using a Coleman 3D potentiometer.

RESULTS AND DISCUSSION

Reduction with 1-amino-2-naphthol-4-sulphonic acid

This reagent was prepared according to the method of King (7). In this procedure 5 minutes is recommended as the optimal time for colour development. From preliminary observations, however, it was evident that the colour continued to increase in intensity for a longer period. The optimal time for colour development was studied using 3 concentrations of phosphorus. The final pH in this method was found to be approximately 0.5.

From the results given in Table 1 it is evident that the colour develops rapidly during the first 10 minutes. After this period the rate of colour development was greatly reduced but it continued at a uniform rate throughout the period of observation. These observations are in general agreement with the results obtained by Gomori (6). For the study on the effect of acidity a 20-minute period was chosen for the colour development with this reagent. Although Gomori suggested 10 minutes as the optimum time, it was considered that a longer period of time would ensure more uniform results in the present investigations.

In Figure 1 is shown the development of the molybdenum colour at different acidities with two concentrations of phosphorus, as well as in the control to which no phosphorus was added. It can be seen from these data that the acid tolerance in this method using amino-naphthol-sulphonic acid extends over a wide range from pH 0.05 to pH 0.6. In this range the intensity of colour developed in the presence of inorganic phosphate was very uniform, while there was no reduction of the molybdate in the absence

TABLE 2.—DEVELOPMENT OF MOLYBDENUM BLUE COLOUR BY METHYL-*p*-AMINO-PHENOL SULPHATE IN THE PRESENCE OF VARYING AMOUNTS OF INORGANIC PHOSPHATE AT pH 0.7

Mgm. of P in 10 ml.	Photometric reading time in minutes								
	2	5	10	15	20	30	40	50	60
0.006	83.5	82.4	82.2	82.1	82.0	81.8	81.4	81.1	80.8
0.02	61.0	58.0	57.4	57.2	57.0	57.0	57.0	56.8	56.6
0.04	42.0	37.8	36.0	34.8	34.2	34.0	33.8	33.6	33.4

of phosphorus. With increasing alkalinity above pH 0.6, the molybdenum blue colour developed very rapidly in the presence of phosphorus, reaching a maximum at about pH 1.0 to 1.1 and then decreased at a slower rate to a low level at pH 1.55. A comparable increase in colour developed when no inorganic phosphate was added. However, in this range of acidity the quantitative determination of phosphorus would be hazardous, since slight variations in pH would incur extremely large errors. On the other hand, as stated above, very accurate measurements can be made when the acidity in the final solution lies in the range of pH 0.05 to 0.6. Although no data are presented here, it was observed that with acidities greater than pH 0.05 the rate of colour development in the presence of phosphorus is very slow.

Reduction with Methyl-p-aminophenol Sulphate (elon)

This reagent was prepared by dissolving 1 gram of elon in 100 ml. of 3 per cent sodium bisulphite as outlined by Gomori (6). The pH of the final solution in Gomori's method was found to be approximately 0.7. This acidity was used in the preliminary study to check the optimum time for the development of colour in the presence of varying amounts of phosphorus.

The data of this study are presented in Table 2.

The intensity of the blue colour developed rapidly during the first 15 minutes and at a reduced rate to 30 minutes. After this time there was a very slight increase in colour up to 60 minutes. These findings again were in general agreement with those of Gomori (6), differing only in that Gomori found no increase in colour after 30 minutes.

A 30-minute period was used in the study of the effect of pH on colour development with this reducing agent in the presence of two concentrations of phosphorus and when no phosphorus was added to the reagents. From the results of this investigation, presented in Figure 2, it can be seen that satisfactory results were obtained in the range of acidity from pH 0.2 to 0.8. In the blank, containing only the reagents and no added phosphorus, there was no reduction of molybdate from pH 0.05 to pH 0.8; above this pH the molybdenum blue developed very rapidly. In the presence of

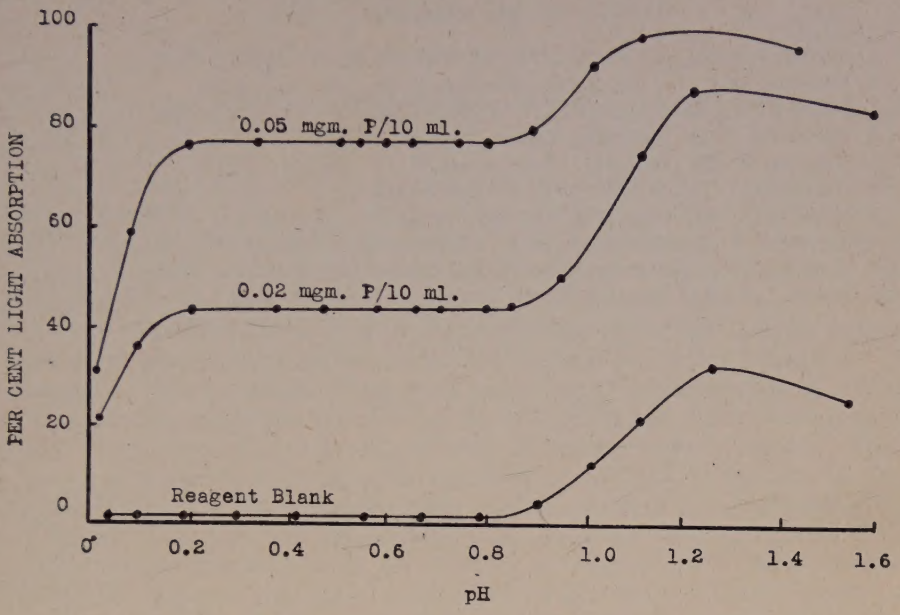

FIGURE 2. Effect of acidity on the development of the molybdenum blue colour by methyl-*p*-amino-phenol sulphate in the presence of inorganic phosphate and in the reagent blank.

added inorganic phosphate the colour developed rapidly from pH 0.05 to about pH 0.2, and thereafter remained constant up to pH 0.8. With greater alkalinity the colour increased again and at a rate comparable to the development of colour in the reagent blank. The acid tolerance of elon is about the same as that of amino-naphthol-sulphonic acid and the range occurs at a slightly higher pH. On the basis of greater stability of colour, elon offers an improvement over amino-naphthol-sulphonic acid as the reducing agent in King's method for the determination of phosphorus.

SUMMARY

A study was made of the effect of varying the concentrations of perchloric acid on the development of the molybdenum blue colour in the determination of phosphorus.

With 1-amino-2-naphthol-4-sulphonic acid the optimum time for the development of the molybdenum blue colour was found to be about 20 minutes, while with methyl-*p*-amino-phenol sulphate (elon) the optimum time was about 30 minutes. Using these periods for colour development, the suitable ranges of acidity in the final solution were found to be pH 0.05 to pH 0.6 for the sulphonic acid and pH 0.2 to pH 0.8 for elon.

Elon gave a molybdenum colour which was more stable than that produced by the sulphonic acid and on this basis appeared to be a more suitable reducing agent in this procedure

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MACROPHOMINA AND FUSARIUM ATTACKING FIELD BEANS IN ONTARIO[1]

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The first record of *Macrophomina Phaseoli* (Maubl.) Ashby (=*Rhizoctonia bataticola* (Taub.) Butler) attacking field beans (*Phaseolus vulgaris* L.) in Canada was obtained in September, 1945, a year after it was reported for the first time on soybean in Canada by Hildebrand *et al.* (5). Following a period of unusually warm weather, mature bean plants from a plot at the Harrow laboratory were found on examination to include a large number with minute sclerotia embedded in the cortical and xylem tissues of the lower part of the stem. This observation led to an inspection of certain fields in the bean-growing district of Kent County, and, from three of these, specimens displaying sclerotia of the organism were obtained. When pieces of tissue containing sclerotia were transferred to potato dextrose agar, cultures of *Macrophomina* were readily obtained. In most instances, however, *Fusarium* was also isolated, the slower-growing mycelium of the latter fungus becoming very conspicuous against the dark background of sclerotia. The plants from which these fungi were isolated showed no marked evidence of disease, but the fact that both *Macrophomina* and *Fusarium* have previously been reported by other authors as attacking field beans made it advisable to establish definitely whether the isolates obtained here were likewise pathogenic. For this purpose greenhouse infection experiments were undertaken. The occurrence of morphologic strains of both fungi among isolates from the various fields was also studied.

DESCRIPTION OF THE DISEASE

The most striking indication of *Macrophomina* infection was the presence in the tissues of abundant small black sclerotia characteristic of this fungus. Andrus (1) employs the term "ashy stem blight" to describe the resulting greyish appearance of the stem, and regards this as characteristic of older plant infections, the term "charcoal rot" being more aptly applied to the black lesions that appear in seedling infections. Sclerotia were commonly found throughout all the sub-epidermal tissues, including the xylem and pith. Often associated with this condition was a vertical splitting at the ground level of the tissues exterior to the xylem and, as illustrated in Figure 1, this led to a characteristic frayed appearance. No foliage symptoms could be connected with the presence of sclerotia, probably because the condition was not noted until late summer when maturation had led to partial or even complete defoliation.

The presence of *Fusarium* was not suspected until it appeared in a large proportion of the tissue plantings. It was frequently isolated from plants unaffected by *Macrophomina*, and in such instances the presence of

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Fusarium was usually correlated with a pinkish discoloration of the xylem. Mackie (9) found that infection of bean plants by *Sclerotium* (= *Rhizoctonia*) *bataticola* was commonly associated with *Fusarium*, the latter fungus being characterized by pink discolorations. A blight of beans in Palestine was shown by Reichert and Hellinger (14) to be caused by *Rhizoctonia bataticola*, usually alone, but sometimes in association with a *Fusarium*. In the present instance, when both organisms were present, the symptoms of *Fusarium* were obscured by the more conspicuous symptoms of *Macrophomina*. The longitudinal shredding mentioned above is not a Fusarium symptom since it was never observed on plants from which only *Fusarium* could be isolated, but it was frequent on those that yielded only *Macrophomina*.

ISOLATIONS FROM DISEASED PLANTS

In studying the occurrence of *Macrophomina* and *Fusarium*, the stems in question were split open and small pieces of xylem tissue removed from the freshly-exposed surfaces were transferred to Petri dishes containing potato dextrose agar. A day or two later *Macrophomina*, when present, had made sufficient growth so that small pieces of agar at the advancing edge of the cultures could be removed to fresh plates, and pure cultures were thereby obtained. The slower-growing mycelium of *Fusarium* advanced inconspicuously throughout the cultures of *Macrophomina* and if the transfers were delayed, both fungi resulted. It was noted that the reverse was not true, *Macrophomina* being unable to invade cultures of *Fusarium*. To separate *Fusarium* from *Macrophomina*, conidial suspensions of the former fungus were prepared from the aerial mycelium at the centre of the combined cultures and streaked on agar plates from which single germinating conidia were removed by the method employed by Miller (10). Commonly four or five single spore cultures were obtained from each *Fusarium* isolate.

In Table 1 are summarized the results of isolations from the four fields in question. A, B, and C represent three commercial plantings approximately 50 miles distant from H, the plot at the Harrow laboratory. All specimens included in the table showed macroscopic evidence of the presence of sclerotia, except those from field H designated with an asterisk. It will be noted that *Macrophomina* was isolated from 85 and *Fusarium* from

TABLE 1.—OCCURRENCE OF *Macrophomina* AND *Fusarium* ON SPECIMENS OF FIELD BEANS AS DETERMINED BY TISSUE PLANTINGS ON POTATO DEXTROSE AGAR

Field	Number of plants from which plantings were made	Results of isolations		
		Macrophomina	Fusarium	Macrophomina + Fusarium
A	25	7	2	16
B	14	3	2	8
C	7	4	0	3
H	45	11	1	33
H	76*	0	53	0

* Specimens without macroscopically-visible sclerotia

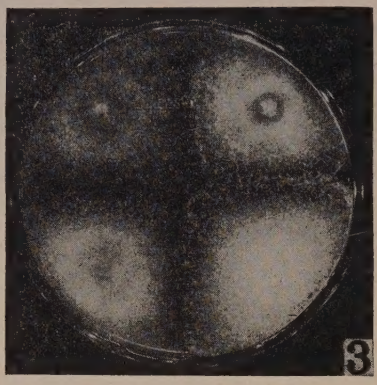

FIGURE 1. Mature bean plants showing longitudinal shredding of outer tissues of stem at ground level, characteristic of infection by *Macrophomina*.

FIGURE 2. Two strains of *Macrophomina* from field H; 26-day-old cultures on potato dextrose agar.

FIGURE 3. Four strains of *Fusarium*, upper two from field A, lower two from field B; 8-day-old cultures on potato dextrose agar.

65 of the total of 91 sclerotia-bearing plants. In 60 instances both were present in the same plant. On the other hand, no isolates of *Macrophomina* were obtained from the 76 plants from field H that displayed no sclerotia, but 53 yielded *Fusarium*. It did not follow, however, that absence of macroscopically-visible sclerotia implied absence of *Macrophomina*, since tissue isolations from above the upper limit of visible sclerotia in affected plants were found to yield cultures of the fungus almost as often as those from the lower part of the same stems where sclerotia were abundant.

The isolates of *Macrophomina* and *Fusarium* failed to show uniformity in cultural characters. In the former, differences in regard to size and abundance of sclerotia were strikingly evident. In Figure 2 are shown two isolations from field H, one of which appears darker than the other owing to the fact that it produced far more sclerotia in culture on potato dextrose agar. Differences in average diameter of sclerotia produced by two isolates from field B are listed in Table 3. Tompkins and Gardner (19) state that no two of the three cultures which they obtained from bean seedlings were alike in cultural characters or sclerotial size. With regard to *Fusarium*,

TABLE 2.—DISEASE INCIDENCE UNDER VARYING CONDITIONS OF SOIL TEMPERATURE IN SOIL INFESTED WITH *Macrophomina* AND *Fusarium*

Pathogen	Emergence in 8 days, %			Mortality in 21 days*, %		
	25° C.	30° C.	35° C.	25° C.	30° C.	35° C.
M-a**	75	80	60	15	40	55
M-b	85	45	55	15	65	55
F-a	60	50	70	30	50	30
F-b	40	20	5	60	80	95
M-a F-a	80	80	70	15	40	55
M-b F-b	45	35	35	55	70	80
Check-a	65	85	85	30	15	15
Check-b	95	95	80	5	10	20

* Estimated by the number of seedlings remaining alive at this time, assuming 100% viability of seed.
** M-a = *Macrophomina*, strain a; etc.

the isolates differed primarily in the appearance of the aerial mycelium, as illustrated in Figure 3, in pigmentation, and in the character and abundance of sporulation. It is not possible to state precisely the actual number of strains isolated since, although in many instances the differences were quite sharp, it was often difficult to be certain that two given isolates were identical, even when grown from single conidia in the same Petri dish. Likewise in *Macrophomina*, differences were often so slight that separation of strains was difficult. It would be safe to state, however, that at least 10 strains of *Macrophomina* and 15 of *Fusarium* were obtained. There were two instances in which the number of *Macrophomina* strains in a given field appeared limited. Field C yielded only one cultural type and field B two, one of which appeared identical with the strain from field C. It is likely that, had more isolates been obtained from these fields, the number of strains would have been increased.

EFFECT OF TEMPERATURE ON DISEASE INCIDENCE

An observation made while examining the bean plants in field A suggested that higher temperatures favoured infection by *Macrophomina*. It was noted that, whereas sclerotia were evident on most of the bean plants on a south slope, not more than 5 per cent of those from an equally steep north slope in the same field showed the condition. The disease was not found on beans growing in heavy clay soil in the same area. While this soil would probably be cooler on the average than the sandy loam soil of the fields in which the disease was found, factors other than temperature might be responsible for its absence. Kendrick (6) concluded that seedling blight caused by this fungus was favoured by a daytime temperature of 95° C. or above in the surface inch of soil; and likewise Tompkins and Gardner (19) found seedling infection to be greatest at higher soil temperatures.

A greenhouse infection experiment was performed in May, 1946, employing three Wisconsin tanks of 8-container capacity each, operated at 25°, 30° and 35° C., respectively. The containers were filled to within 5

inches of the top with sand (not sterilized) following which 4 inches of steam-sterilized sandy loam soil was added to each. The soil in two of the containers was infested with *Macrophomina*, that in two others with *Fusarium*. An additional two contained both *Macrophomina* and *Fusarium*, while the remaining two served as checks. The soil was infested by incorporating, in the proportion of about 1.5% by volume, cornmeal-sand medium on which the fungi had grown for 10 days. The two strains of *Macrophomina* employed were first purified by the hyphal-tip method, and the two *Fusarium* strains were purified by obtaining a monoconidial culture of each. Twenty bean seeds (variety Michelite) were planted in each container. All containers received uniform and moderate amounts of water.

The results of the temperature experiment are summarized in Table 2. The general trend with both fungi was an increase in disease incidence toward the higher temperatures. This is not evident from the figures in the case of the milder *Fusarium* strain (F-a), but an examination of the hypocotyl bases revealed that the lesions were more extensive at 35° C. than at 30° or 25° C., and the plants were less healthy on the whole at the highest temperature. An increase of disease incidence with temperature was also noted where the two pathogens were mixed. Some mortality was noted in the checks but this did not increase at the higher temperatures. Isolations attempted from 30 plants growing in the Macrophomina-infested soil yielded this fungus in 24 instances. From 24 plants in the Fusarium-infested soil *Fusarium* was isolated in 18 instances, but 32 plants from the check cans failed to yield either of these fungi.

The seedling symptoms caused by both *Macrophomina* strains were identical and resembled closely those described and figured by Kendrick (6) and by Tompkins and Gardner (19). The first sign of infection was the appearance of small, irregular, black areas on the cotyledons, which tended to coalesce. Within a week after the first appearance of the lesions, entire cotyledons became blackened, and black streaks began to spread up and down the stem from the point of attachment of the cotyledons. Sometimes another black streak extended up from an infected area at the base of the hypocotyl, although infection at this point appeared less frequent than through the cotyledons. Death of the seedlings ensued if the "charcoal rot" penetrated all or most of the way through the hypocotyl. Tissue plantings from blackened areas consistently yielded the fungus.

TABLE 3.—SIZE OF SCLEROTIA OF 2 STRAINS OF *Macrophomina* FROM FIELD B ON BOTH NATURAL AND ARTIFICIAL SUBSTRATA

Strain	Substratum	Number of sclerotia measured	Average dimensions (microns)
B1	Stem of naturally infected plant	100	93.3 × 81.1
B1	Peripheral zone of 20-day-old Petri dish culture	100	108 × 92.6
B3	Stem of naturally infected plant	100	73.2 × 62.2
B3	Peripheral zone of 20-day-old Petri dish culture	100	84.5 × 69.1

Both strains of *Macrophomina* seemed equally virulent in this experiment. However, in an additional greenhouse experiment performed with 7-inch pots in June, 1946, employing these and four additional strains, one of the latter seemed much more virulent than any of the others, causing more rapid killing on the average. Symptoms were similar to those noted in the temperature experiment.

Seedlings growing in the containers infested with *Fusarium* showed no black lesions and invasion via the cotyledons was evident in only one instance. The characteristic effect of attack by *Fusarium* was the appearance of a brownish discoloration at the base of the hypocotyl which sometimes extended upwards for a short distance. Isolations from such affected areas consistently yielded cultures of the fungus. Also noted was a preemergent killing by one of the strains (F-b) which was progressively more severe at the higher temperatures, as indicated in Table 2. The affected seeds displayed a web-like covering of mycelium and their development seldom exceeded a limited elongation of the radicle. This strain was more virulent than the other (F-a), causing much higher mortality.

The plants growing in the containers infested with both fungi showed essentially a combination of the symptoms described above for the separate inoculations, but the dark lesions caused by *Macrophomina* were the more conspicuous. From Table 2 it is apparent that disease incidence was more severe at the higher temperatures. While it might be expected that infection by both organisms would be more severe than that from either alone, the evidence summarized in the table does not support this view, since mortality was not in any instance greater than that caused by single infection with the more virulent of the two at the same temperature. However, when the more virulent strain of *Fusarium* (F-b) was included in the combination, mortality was greater than when the milder strain (F-a) was included. In his studies on cotton root rot in India, Prasad (13) found *Rhizoctonia bataticola* and a *Fusarium* to be capable of causing a disease of cotton similar to root rot, but in this instance the fungi were more parasitic when together than either of them singly.

Macrophomina IDENTIFICATION OF THE PATHOGENS

In cultural characteristics, and in the appearance of the sclerotia on the host, this fungus seemed identical with that previously found in Ontario on soybean and identified by Hildebrand *et al.* (5) as *Macrophomina Phaseoli* (Maubl.) Ashby. Although pycnidia were not obtained in the present instance, only the sclerotial stage, *Rhizoctonia bataticola* (Taub.) Butler being found, nevertheless, similarities both in morphology and symptomology to a fungus described by previous writers as attacking beans and connected by them with the pycnidial stage support the foregoing identification.

Regarding morphology, measurements of sclerotia produced in culture, as well as on the host, were found to be within the range (less than 120 u) characteristic of Haigh's "C" group (3). In Table 3, measurements of sclerotia from the two strains isolated from field B are given, these representing the approximate extremes of variation in sclerotial size noted among the isolates. It is interesting to note that strain B1 produced larger

sclerotia than B3 on the host as well as in culture, but the average measurements were always less than 120 u. Haigh found that pycnospores always gave cultures belonging to the "C" group, and beans were one of the hosts on which pycnidia were produced.

The symptoms noted in the present instance were similar to those described on lima beans by Andrus (1), both in the seedling (charcoal rot) and mature plant (ashy stem blight) stages. He observed the causal fungus to produce sclerotia and pycnidia characteristic of *M. Phaseoli.*

Fusarium

The taxonomy of *Fusarium*, in contrast to that of *Macrophomina*, has generally been characterized by a tendency to draw fine distinctions between species. Thus while the morphologically variable isolates of *Macrophomina* may be placed with confidence in the species *M. Phaseoli* (Maubl.) Ashby, the isolates of *Fusarium* in view of their lack of uniformity cannot be assigned to any single species. There is the further difficulty that, as Miller (11, 12) has indicated, owing to certain techniques that have been employed in Fusarium taxonomy, many of the species descriptions are based on the characteristics of cultural variants of the isolates studied.

The extremes of the range of variability of the isolates are represented approximately by the two strains F-a and F-b employed in the temperature experiment. These are illustrated in Figure 3 (upper left and upper right, respectively). Both were isolated from field A, although from different plants, and were shown to be pathogenic to bean seedlings in the experiment described, F-a being somewhat less virulent than F-b. In the photograph it is apparent that F-b produced on potato dextrose agar more aerial mycelium than F-a. By far the greater number of the isolates were of the "fully raised" type, resembling F-b rather than F-a. Sporulation in F-a was profuse, taking the form of a slimy layer overlying the surface of the agar, a so-called "pionnotes", consisting almost entirely of macroconidia. The conidia of F-b were less abundant and only about 4 per cent were macroconidia, the majority being the small nonseptate macroconidia. The macroconidia of F-a had blunt ends, characteristic of the section Martiella, but those of F-b were more pointed as in the section Elegans. Thus, were the taxonomic revisions proposed by Snyder and Hansen (16, 17) employed, it would still be difficult to know for certain in which species to place the Fusaria studied here, since they group the members of sections Elegans and Martiella in two species, *F. oxysporum* and *F. solani*, respectively. In view of the present unsettled state of the taxonomy of the genus it seems unwise to attempt an identification on morphological grounds.

With regard to symptomology, Mackie (9) noted pink discolorations on plants affected by a wilt *Fusarium* that was commonly associated with *Macrophomina*, indicating a resemblance to the pathogen studied here, but it is questionable whether either is the same as the vascular *Fusarium* described by Kendrick and Snyder (8) as causing yellows of bean. The latter fungus caused a dark-brown discoloration of the vascular system extending into the lateral branches and petioles, in contrast to the mild pinkish discoloration noted here. The symptoms of dry root rot of bean

as described by Burkholder (2) were unlike those found in the present instance on field specimens, although the brownish discoloration and rotting of the hypocotyl bases of seedlings noted above in the temperature experiment resembled dry root rot. Snyder (15) states that the Martiella *Fusarium* responsible for this disease may discolour the vascular elements following decay of the cortical tissue, but here the field specimens with vascular discoloration did not show evidence of cortical decay. It may therefore be concluded that, on the data available, the Fusarium disease studied here does not seem identical with either dry root rot or Fusarium yellows of beans.

DISCUSSION

The discovery, after a period of unusually warm weather, of ashy stem blight on field beans and experimental confirmation of the favourable effect of high soil temperatures on the disease indicates that the latter may be a factor in bean production in Ontario during summers when temperatures are above normal. Harter and Zaumeyer (4) state that it has caused losses as high as 60 to 65 per cent in some fields in Mississippi. In the southern States it may be severe throughout the entire growing season. Kendrick (6) found it to be particularly severe on young seedlings in California following periods of unusually high temperature, and sometimes many of the seedlings were killed before reaching the surface of the soil. The predominant symptom on seedlings is a pronounced blackening of the affected parts, and Andrus (1) and Young (20) employ the term "charcoal rot" to describe this expression of the disease. The isolates of *Macrophomina* studied here were shown to be capable of causing the seedling phase of the disease, and it is therefore possible that this could occur in Ontario should high soil temperatures prevail following planting. In addition, the temperature experiment indicated that attack by *Fusarium* would likewise be favoured by high temperature.

Since the plot at the Harrow laboratory had not previously been planted with field beans, it is not easy to understand how such a high proportion (20 per cent) of the plants became infected with *Macrophomina*. Harter and Zaumeyer (4) point out that the fungus has a wide host range and hence it may have been present on preceding crops. Tompkins and Gardner (19) found isolates of *Macrophomina* from a wide range of hosts to be pathogenic to bean seedlings. Tehon and Boewe (18) found *Macrophomina* on a number of common weeds in Illinois. The possibility of introduction on seed should not be overlooked since Andrus (1) showed it to be seed transmitted. Surface sterilization of lima bean seed did not prevent development of the fungus.

With regard to *Fusarium*, the plot in question had actually been infested before planting with a *Fusarium* isolated the preceding summer from bean, and also with a cultural variant of this fungus, with a view to comparing relative survival under field conditions. Although the cultural variant was not reisolated, the isolates obtained showed a certain diversity and few seemed identical with the original culture. This variation could be accounted for by mutation in the field, by the pre-existence in the plot of strains of *Fusarium* capable of attacking bean, or by seed transmission. Kendrick (7) has shown Fusarium yellows of bean to be seed transmissible and in this instance seed treatment was highly effective.

SUMMARY

Macrophomina Phaseoli (Maubl.) Ashby was found attacking mature bean plants following a period of unusually warm weather. Cultures of this organism were readily obtained from affected plants by making tissue plantings, and *Fusarium* also developed from more than half of the plantings. A cultural study of the isolates revealed that both *Macrophomina* and *Fusarium* included a number of morphologic strains. Both fungi were shown to be pathogenic to bean seedlings, attack being favoured by high soil temperature. Mixed inoculations with the two pathogens were not more virulent than either singly.

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"SMALL BITTER CHERRY", A FRUIT ABNORMALITY OF THE BING CHERRY VARIETY[1]

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An abnormality in the fruit of Bing Cherry trees has been observed for several years in the Okanagan Valley of British Columbia. The trouble is of some intrinsic interest, but of importance only because it can easily be confused with little cherry (1), a serious virus disease. The name "small bitter cherry" has been used, and is suggested for the disease herein described.

The diagnosis of "small bitter cherry" is possible only during the picking season, for the growth and productiveness of affected trees appear to be normal. The fruit set of these trees is likewise normal, but the fruit produced is of two distinct types with few intergrades. (Figure 1A). Some of the cherries are normal in size, shape, colour, taste, and date of maturity. Others are less than half normal in size, regular in shape, oval in side view, and round when viewed from the distal end. At picking time, they are the bright red of an immature cherry and are somewhat bitter, with an objectionable flavour reminiscent of the smell of stagnant ditch water. Later in the season, they are less bitter and taste somewhat fermented. They may hang on the tree for a month or more after picking time without ripening. These small cherries may be few in number or they may constitute more than half the crop. Usually, normal and small cherries occur together in the same part of the tree, but on some branches the cherries are all small, and on others they are all normal. In one typical tree, normal and small cherries showed volumes of 8.2 cc. and 3.0 cc., and soluble-solid contents of 24.3 per cent and 12.6 per cent, respectively.

"Small bitter cherry" has been observed in eight trees in five orchards. It was first noticed in a single Bing tree at Oliver in 1940. It was reported that this tree had produced poor fruit since 1937, and that one other similar tree had been removed. In 1946 two other Bing trees in the same orchard showed "small bitter cherry". One, slightly affected, was reported to be showing abnormal fruit for the first time. The other, affected through much of one side, was reported to have produced abnormal fruit since 1944. Lambert trees in this orchard have remained normal. In 1944, two affected Bing trees were found in another orchard in Oliver. These trees were reported to have been affected for some years, while other Bing and Lambert trees in the same orchard remained normal. In 1946, surveys showed one affected Bing in each of three orchards, one in Oliver and two in Osoyoos. One was affected throughout, with deep suture also present, and two were affected in part. One was reported to be affected for the first time, and two to have had abnormal fruit for at least one year previously.

The cause of "small bitter cherry" has not been determined. In 1941 and 1942, buds and scions from the affected tree found in 1940 were placed on eight young trees—six Bing, one Lambert, and one Napoleon. Tissue

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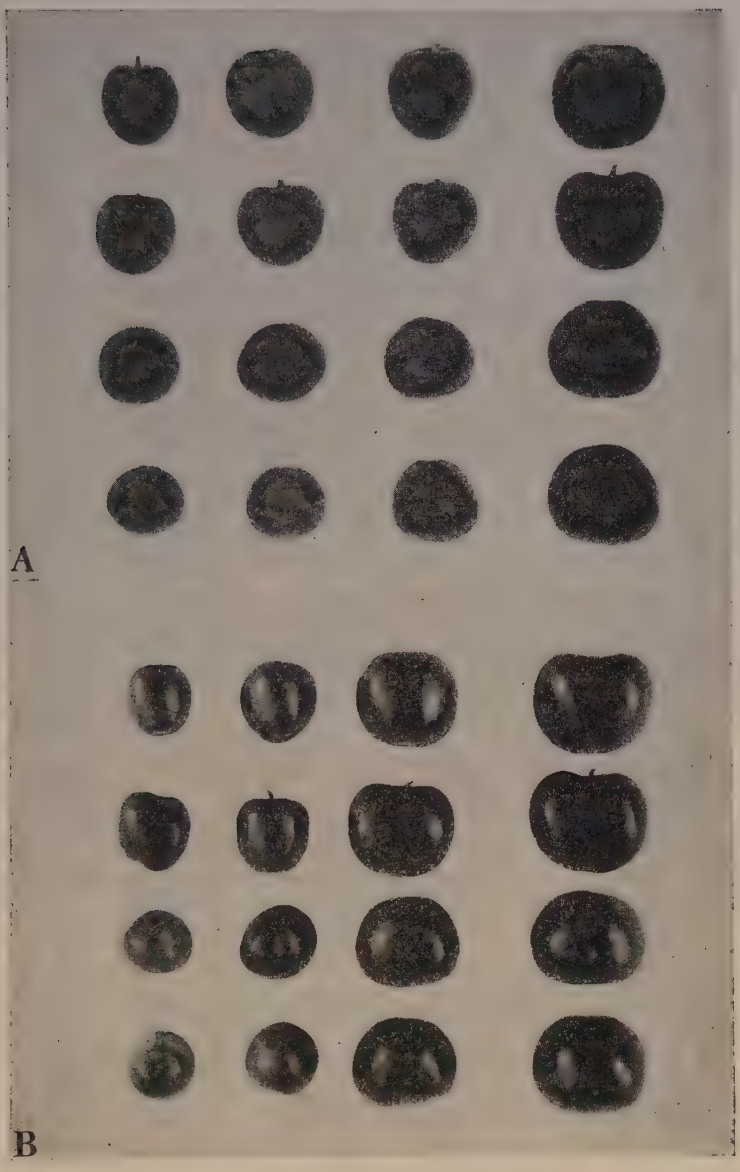

FIGURE 1. Fruits of the Bing Cherry.

A. Sixteen fruits from tree affected with "small bitter cherry".
B. *Left*, twelve fruits from tree affected with little cherry.
Right, four normal fruits.

union was obtained in all trees. In 1943, they produced a small amount of fruit which was normal except for some chill effect. They bore normal fruit again in 1944. Two of them, one Bing and one Lambert, were retained and were still bearing normal fruit in 1946. In a further transmission experiment in 1944, buds from one of the affected trees found that year

were placed on two young trees, one Bing and one Lambert. Union was obtained in both trees and they both bore normal fruit in 1946. Transmission, therefore, has not been obtained.

Though "small bitter cherry" appears to be of small economic importance, it is of particular consequence to cherry growers in the Okanagan due to the similarity of its symptoms to those of little cherry. This latter is a very serious virus disease which has spread with exceptional rapidity through the Kootenay area and which, it is feared, might at any time reach the Okanagan.

While the symptoms of these disorders are similar in many respects, there are certain characteristics that serve to differentiate between them. "Small bitter cherry" is only known to occur on the Bing variety, while all commercial varieties are susceptible to little cherry. In shape, "small bitter cherry" fruit is oval in side view and round when viewed from the distal end, while little cherry fruit is tapered in side view and triangular from the end view. The fruit on trees having "small bitter cherry" falls into two distinct classes, altogether normal, and typical "small bitter cherry", with few intergrades, while the fruit on a Bing affected with little cherry ranges from normal to typical little cherry, with many intergrades. In taste, a "small bitter cherry" fruit is bitter and even objectionable, and the normal sized fruit on the same tree has natural sweetness and flavour, while on a tree affected with little cherry all the fruit is flat and off-flavour, even though it may be normal in size and colour. In "small bitter cherry", some branches have all normal fruit and others carry only small cherries. In little cherry, the cherries are seldom all small on any one branch, and branches with all normal fruit are likely to be observed only during the early stages of infection.

Finally, "small bitter cherry" has not been transmitted to healthy trees through tissue grafts, and natural spread, if it occurs at all, is very slow. Little cherry, on the other hand, is easily transmitted, and the natural spread is extraordinarily rapid.

SUMMARY

1. An abnormality of the fruit of Bing cherry is reported and the name "small bitter cherry" suggested.

2. Symptoms are confined to the fruit. Tree growth and productiveness are not affected.

3. On affected trees, fruit is almost entirely of two grades, normal and typical "small bitter cherry".

4. A "small bitter cherry" is oval in side view, round in end view, is still bright red at normal picking time, and is less than half the size of the normal fruit.

5. The disorder has not been transmitted by tissue grafts to healthy trees.

6. This disorder is not of great economic importance in itself, as few affected trees have been found; its importance lies in its possible confusion with little cherry. The distinctive characteristics of the two are presented.

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LENGTH OF CUTTING SEASON OF ASPARAGUS

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The problem of the length of time or date to which asparagus plantations may profitably be cut each year has received the attention of several workers. Lloyd and McCollum (3) in Illinois found that cutting the first year after planting was not profitable and concluded that only a four week cutting season during the second year was beneficial. Cutting for eight weeks after the fourth year was found to produce a greater total yield than cutting for only six weeks. Haber (2) concluded that cutting to June 15 (approximately seven weeks) under Iowa conditions was the most profitable. Plots cut until July 15 (eleven weeks) died out after six cutting seasons. He has also shown that a short harvesting period produces the heaviest stalks, although as the season progresses the cut stalks become smaller. Deonier and Hoffman (1) found that, although asparagus production in Mississippi was not commercially profitable, harvesting between four and eight weeks in the spring produced the highest yields. Jones (4) on the other hand found that there was little difference in yield between plots cut for ten or twelve weeks in California. In fact, those plots cut for twelve weeks outyielded those cut for ten weeks by 10 per cent over a six-year period. The above references show that the best length of cutting season for asparagus must be determined for limited conditions.

MATERIALS AND METHODS

Uniform one-year-old Mary Washington asparagus plants were set out in the spring of 1932 on a Monroe clay loam at the Dominion Experimental Farm, Agassiz, B.C. The area used had been in pasture for many years prior to planting. Each plot contained twenty plants, spaced 1.5 feet apart in the row, with the rows 5 feet apart. The whole area consisted of four ranges with six 1/290-acre plots in each range. Cutting was commenced in 1935. During 1935 and 1936, all plots were cut uniformly for a period of eight weeks. Upon analysis of this initial data it was found that at least eight, or, preferably twelve replications of any one treatment would be needed to produce significant results. Twelve replicates of two treatments were therefore used, each treatment being replicated three times in each of the four ranges. These treatments consisted of cutting for a period of eight and twelve weeks, as from the first harvest date each year. All plots received manure at the rate of fifteen tons per acre each spring and had similar cultivation throughout the duration of the experiment.

Each plot was cut three times per week and the stalks were counted and weighed immediately after cutting. No other attempt was made to grade the cut stalks. An exceptionally severe frost in the spring of 1943 (−4° F., January 21) was the cause of the abnormally low yields during that year.

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TABLE 1.—MEAN YIELD OF ASPARAGUS IN POUNDS PER PLOT (12 REPLICATES) CUT FOR 8 AND 12 WEEKS

Year	Cut for 8 weeks	Cut for 12 weeks	
		First 8 weeks	Total 12 weeks
	Column A	Column B	Column C
	lb.	lb.	lb.
1935	2.5	2.7	—
1936	6.6	6.7	—
1937	8.8	8.2	13.9
1938	15.0	11.0	16.6
1939	19.8	13.0	18.2
1940	22.6	13.2	19.9
1941	24.0	13.6	20.1
1942	26.8	12.9	17.7
1943	19.5	10.8	14.9
1944	27.4	15.0	21.9
1945	26.5	12.5	16.9
1946	19.2	9.5	13.0
Mean*	20.9	11.9	17.3

* Excluding 1935 and 1936.

Necessary Difference (P = 0.05) between means:
Columns A and B = 2.16 SE (m) = 4.2%
Columns A and C = 0.75 SE (m) = 1.3%

Necessary Difference (P = 0.05) for interaction:
Columns A and B = 1.87 SE (m) = 4.2%
Columns A and C = 2.84 SE (m) = 5.5%

RESULTS

The dates upon which cutting started each year were as follows: 1937, 1938, 1939, Apr. 17; 1940, April 8; 1941, April 2; 1942, 1943, April 11; 1944, April 15; 1945, May 1; and 1946, April 25.

The mean yields in pounds per plot of twelve replicates are presented in Table 1. The yields of the first eight weeks of the twelve-week plots are also shown.

This table shows that cutting asparagus for a period of twelve weeks reduces the vigour of the plants to such an extent that after five years the yields are significantly decreased below those of plants cut for a period of only eight weeks; also peak production is maintained over a shorter period with a twelve-week cutting season than with an eight-week cutting season. It will be noted that with a twelve-week cutting period decline in production commenced with the 1942 harvest and proceeded (with the exception of the 1944 harvest) to decline steadily. With the eight-week cutting season, however, production increased each year (with the exception of 1943) until 1944 and 1945. Decline in production did not commence with the eight-week cutting season until 1946.

The mean weight per stalk calculated from the gross number and weight of stalks harvested per plot each year is presented in Table 2.

TABLE 2.—MEAN WEIGHT OF ASPARAGUS STALKS IN OUNCES (12 REPLICATES) CUT FOR 8 AND 12 WEEKS PER YEAR

Year	Cut for 8 weeks	Cut for 12 weeks	
		First 8 weeks	Total 12 weeks
	Column A	Column B	Column C
	oz.	oz.	oz.
1935	0.80	0.86	—
1936	.73	.74	—
1937	.73	.74	0.69
1938	.76	.72	.67
1939	.91	.86	.84
1940	.95	.89	.82
1941	.97	.94	.97
1942	.89	.76	.71
1943	.94	.99	.91
1944	.68	.74	.68
1945	.73	.69	.67
1946	.53	.54	.54
Mean*	0.81	0.79	0.75

* Excluding 1935 and 1936.

Necessary Difference (P = 0.05) between means:
Columns A and B = not sig. SE (m) = 3.75%
Columns A and C = 0.027 SE (m) = 1.11%

Necessary Difference (P = 0.05) for interaction:
Columns A and B = 0.039 SE (m) = 1.72%
Columns A and C = 0.073 SE (m) = 5.13%

It will be noted that there is no significant difference between the mean weight of stalks cut from the eight-week plots and those cut during the first eight-week period from the twelve-week plots. Those cut from the twelve-week plots are, however, slightly lighter. When the mean weights of the stalks for the whole twelve-week period are computed, it will be seen that they are significantly smaller than those of the eight-week plots. The stalks cut during the last four-week period are, therefore, much lighter than those cut earlier in the season. On the whole there is a greater difference in stalk weight due to season than due to treatment.

DISCUSSION AND CONCLUSIONS

From an examination of the results presented, it is clear that cutting asparagus for a twelve-week period in the coastal area of British Columbia is not so profitable and, in fact, produces less total weight and smaller stalks than are obtained when cutting is stopped after eight weeks. These results in general have been borne out elsewhere. They also show that not only does an eight-week cutting period produce more asparagus but production is maintained at a higher level for longer than is found with plots cut for twelve weeks. Evidence is not available under British Columbia conditions of yields obtained with four- and six-week cutting periods.

The results of a ten-year experiment on cutting asparagus for eight weeks and twelve weeks have shown that:

1. Plots cut for a twelve-week period each year produce significantly less asparagus after five years than do plots cut for eight weeks.
2. The ten-year yield of the twelve-week plots is less than that from the eight-week plots.
3. Decline in production is brought about more rapidly when cutting is extended over a twelve-week period.
4. The size of the stalks from the twelve-week plots is less than from the eight-week plots, although for the first eight weeks the size of stalks from both plots is nearly the same.
5. There is a greater difference in size of stalk due to seasonal variation than there is due to treatment.

ACKNOWLEDGMENT

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METHYL BROMIDE FUMIGATION OF PLANT PRODUCTS IN STEEL BARGES AND THE HOLDS OF SHIPS[1]

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The programme initiated by the Dominion Department of Agriculture for the control of the large populations of insects found in imported plant products was described in a previous paper (6) in which a full account was given of the employment of steel railroad box cars for fumigation purposes. In the pursuance of this programme the need was soon encountered for the utilization of water carriers as fumigation structures, when these could be more economically employed for the transportation of freight.

The fumigant employed in this work was again methyl bromide, and it is therefore not necessary in this paper to repeat the descriptions of the properties of this compound and the general methods of its application, as these were fully given in the previous contribution.

The object of the present paper is to describe the extension of the fumigation technique to ships and barges, a development which might logically be expected to follow the successful use of methyl bromide in warehouses and railroad cars.

PENETRATION AND SUBSEQUENT VENTILATION OF THE FUMIGANT

When the possibility of treating the commodities in the holds of barges and ships was first considered, it was found that few data were on hand to indicate the maximum effective depth of penetration through piles of bags containing stored products which could be expected, with the application of normal fumigation doses of one to two pounds of methyl bromide per thousand cubic feet. Piles of bags fumigated in warehouses are usually less than 15 feet high and the lengths of the sides of the piles rarely exceed 15 feet on any side. Therefore, in order to reach the centres of these large piles, the fumigant would only have to travel a maximum of 7.5 feet from the outside. Also, in railroad cars the bags are never piled more than 8 feet high. Bellemare (2) reports a successful penetration of a large single pile of bags containing cocoa beans, with dimensions 60 feet long, 50 feet wide and 22 feet high during a warehouse fumigation to control the Indian Meal moth, *Plodia interpunctella* Hbn., using methyl bromide at the rate of 1.5 pounds per thousand cubic feet for 24 hours at temperatures of 80° to 90° F. It was considered that toxic concentrations of the fumigant would have to travel at least 22 feet to reach insects at the middle of the pile.

The question as to whether methyl bromide could penetrate the deep layers of bags in the holds of ships could not, therefore, be answered off-hand. The work here described has indicated further depths of penetration through piles of jute and cotton bags containing peanuts, but the final answer to this problem can only be determined in the light of future experi-

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FIGURE 1. Standard type Mississippi barge

ence in practical fumigation operations, and answered finally for each particular type of commodity by gas analysis through chemical means, or when failures in control due to lack of penetration are encountered.

It must also be remembered that, in undertaking fumigation in holds of ships and similar spaces, due consideration must also be paid to the question of adequate ventilation following exposure, so as to present no hazard to those handling the goods or travelling on any vessel concerned. This matter is of special importance when the only egress to the outside air is through the open hatch covers, so that no draught of air can be arranged, thus leaving a "dead" space at the bottom of the holds from which the fumigation concentrations can only be dissipated by upward diffusion.

Use of the Halide Leak Detector. Throughout the operations described in this paper, the method employed for the detection of residual concentrations and leaks of methyl bromide was the extensive use of the methyl alcohol lamp known and marketed as the "Halide Leak Detector". The pure methyl alcohol in the reservoir of this lamp is warmed by priming with a small amount of alcohol ignited in a cup under the burner. The alcohol vapour under pressure burns with a blue tinted, almost invisible flame when released from the reservoir, ignited in the burner, and mixed with air drawn from a sampling tube. If any gaseous halide is present in the air sample, it breaks down on coming in contact with the heated copper flame ring in the burner, and turns the flame to various intensities of green merging into bright blue according to the amount of halide present. This test is, therefore, not specific for methyl bromide if other halides are present, but it is of value as a guide under practical fumigation operations with this particular fumigant. According to a publication of the United States Department of Agriculture (5), this lamp is sensitive to concentrations as low as 40 parts per million of methyl bromide, and a table is published

showing the flame colour to be expected at different concentrations up to 800 parts per million. Armitage and Steinweden (1), of the California Department of Agriculture, indicate that it is only accurate as low as 50 parts per million.

In the work described in this paper, the lamp was used principally to determine if it was safe for the crews to reoccupy their quarters or for men to work at unloading the fumigated cargo.

It is not proposed, in this paper, to discuss the value, from the point of view of human safety, of the reading given by the Halide Leak Detector. However, it must be pointed out that the conditions prevailing in a fumigated structure cannot be compared with those in a factory or industrial plant where methyl bromide is being manufactured or where cylinders and other containers are being filled. Workers in the latter types of establishment need constant protection, week in and week out, as the supply of methyl bromide leaking out into the working space may be constantly renewed from a source of leak involved in the nature of the process itself. On the other hand, following a successful fumigation and aeration of a commodity and the structures containing it, no further gas is applied, and there is no possibility of a state of chronic poisoning arising among the inmates of such a place. In the course of ten years' experience with this chemical—much of it involving observations on the practical results of large scale fumigations—the writer has no record of any mishap or sickness reported in fumigated structures following an adequate aeration, as demonstrated by the absence of any green colour reaction in the flame of a properly functioning detector of the type described. In fumigation work, the chief danger is to the fumigators themselves, involved either in the application of the chemical or during the period of opening up, or to other persons accidentally exposed to toxic concentrations during the fumigation or aeration periods.

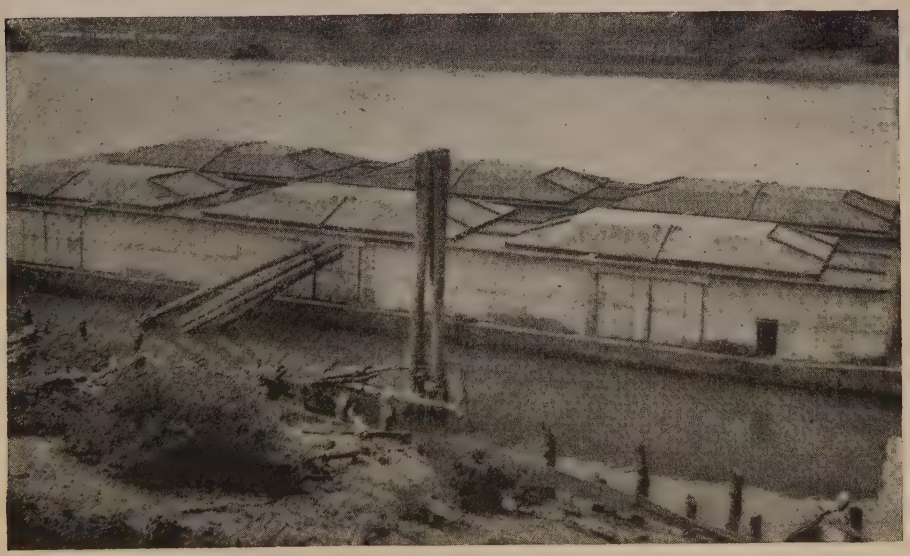

FIGURE 2. Kelly type Mississippi barge

In the work described here, complete absence of any green reaction in the flame in the free air of any given space was taken as the safety point, and was required before any person was permitted to stay or work therein.

FUMIGATION IN STEEL BARGES[1]

The use of ethylene oxide for the fumigation of dried fruits in steel barges has been described by Brown (3). In a recent paper, Brown and Lewis (4) record gas concentrations found by chemical analysis during the fumigation with methyl bromide and hydrogen cyanide of steel barges containing empty bags. No insects were mentioned as being found in this material, and therefore no record of actual mortality was obtained. It was concluded that methyl bromide would prove very suitable for this type of fumigation.

In the spring of 1945, Argentine peanuts in cotton bags consigned to Hamilton, Ontario, Canada, for crushing were unloaded at New Orleans, Louisiana, and moved in five river barges via the Mississippi water route to points near Pittsburgh, Pennsylvania. Here they were transferred to freight cars for the balance of the journey to Canada. Following the request of this Department, inspectors of the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine, examined this material while being unloaded at New Orleans, and reported a moderate infestation of the following species of insects:

Plodia interpunctella, Hbn., the Indian meal moth.

Ephestia sericarium, Scott = *kuehniella*, Zell., Mediterranean flour moth.

Tenebroides mauritanicus, Linn., the Cadelle.

Oryzaephilus surinamensis, L., saw-toothed grain beetle.

Tribolium castaneum, Hbst., Red flour beetle.

It was considered that the population would be increased considerably during the voyage of 4 to 5 weeks up the Mississippi, and this prediction was subsequently borne out. Arrangements, therefore, were made to treat the barges at the point of transfer to the railroad car. The fumigations were conducted by a firm of pest control operators working under the supervision of officials of the United States Bureau of Entomology and Plant Quarantine, and, during the first fumigation, a representative of this Department.

Description of Barges. The first barge to be subjected to fumigation was of the standard type, illustrated in Figure 1. The roof of the barge, except for about 20 feet at each end, is composed of adjoining steel sections which are connected with overlapping strips to prevent rain seepage. These sections are lifted in and out of place by cables attached to cranes. The barge can be rendered air-tight by sealing with suitable materials at the points of junction of the sections. The barge in question had a capacity of 50,000 cubic feet and contained 9515 bags loaded to depths of 6 to 9 feet.

[1] The section of this paper dealing with barge fumigation is based, firstly, on the observations of Dr. H. E. Gray, Officer in Charge, Stored Product Insect Investigations, Division of Entomology, Ottawa, who undertook to supervise this work at Pittsburgh, Pa. and, secondly, on the reports kindly submitted by officers of the United States Department of Agriculture, Bureau of Entomology and Plant Quarantine. (See further remarks under "Acknowledgments").

The four succeeding barges, which were all of the "Kelly" type, employed an extensive superstructure as shown in Figure 2 and had cubic capacities varying from 30,000 to 50,000 cubic feet. The bags in these barges were piled to a depth of 12 feet.

Sealing Methods. In the first treatment of the "standard" type barge, masking tape was used as the sealing medium. In view of the failure of this fumigation, due partly, it would appear, to poor sealing, greased Kraftex paper was subsequently used on all types of barges, supplemented by the use of asbestos caulking compound for the larger openings. The Kelly barges, as a result of their extensive superstructure, were particularly difficult to seal and a great deal of time and labour was expended in this work.

Application of Fumigant. In the first fumigation of the standard barge, methyl bromide was applied at the rate of one pound per thousand cubic feet for a 20-hour exposure. The commodity and air temperatures were 65° F. and the fumigant was applied from cylinders under pressure, each cylinder being equipped with a three-way outlet so that it was possible to convey some of the gas to both of the extreme ends of the barge, as well as to provide even distribution throughout. Small office type fans were used to aid circulation of the fumigant.

As noted above, the first fumigation was a failure and in subsequent treatments the method of sealing was improved and the dose increased to two pounds per thousand cubic feet, with an exposure period of 40 hours. In later fumigations, the temperatures ranged as high as 80° F. in the commodity and 92° F. in the free air. At the time of ventilation in the later fumigations, a Halide Leak Detector of the acetylene burning type showed flames of a pronounced blue-green colour, demonstrating that there were still considerable concentrations of methyl bromide present. The circulating fans were operated for two hours following the removal of the hatch covers or superstructures to aid ventilation. In the case of the standard barges, ventilation was rather rapid as all the covers could be removed and the entire top surface of the cargo exposed to the air, with the exception of the ends of the barge, permanently covered by 20 feet of deck.

Results. In the first fumigation only about 50 per cent of the adult insects were killed. In view of subsequent experiences, it is believed that this was due to insufficient sealing and inadequate dose, rather than to a short exposure period. The comparatively low dose of 1 pound per thousand cubic feet was apparently not enough to compensate for the leakage which occurred.

All the five succeeding treatments, both with the "standard" and "Kelly" types of barge, were reported by the United States inspectors to be completely successful against the adults and larvae of the five species of insects concerned, thus demonstrating the practicability of this type of treatment.

SCHOONER FUMIGATION

In August, 1946 arrangements were made for the use of diesel "schooners", of the type employed in the lower St. Lawrence river trade, for the movement of part of a cargo of Nigerian peanuts in bags destined

FIGURE 3. St. Lawrence River diesel type schooner

for the crushing plant at Hamilton, Ontario, involving a voyage up the St. Lawrence Canal and across Lake Ontario. The peanuts were heavily infested with populations of the red flour beetle, *Tribolium castaneum*, Herbst. and the saw-toothed grain beetle, *Oryzaephilus surinamensis* L.

Description of Vessels. One of the schooners employed is illustrated in Figure 3. These schooners have wooden hulls, weigh about 114 tons unloaded, and are driven by diesel engines. The single hold has a length of 60 feet and a maximum beam of 27 feet with a capacity of approximately 16,000 cubic feet, but in computing the dose of fumigant, allowance has to be made for leakage into the entire engine room space, as the partition between is usually extremely flimsy. The holds when loaded took 1500 bags of Nigerian peanuts, with a total weight of 150 tons, packed 11 bags deep at the middle of the hatch, giving a depth of pile of 12 feet. In the rest of the hold the maximum depth of the pile was 10 feet. This cargo, therefore, consisted of a solid pile of bags with approximate dimensions of $60 \times 27 \times 10$ feet, with the sides and bottom pressed heavily against the inside of the hull of the ship.

Sealing Methods. Each boat had one or two hatch covers, which were sealed first by running strips of Kraftex paper glued with flour paste across the junction between the individual boards. These were then covered with widths of tar paper sealed together with Kraftex paper and flour paste. (Figure 4). The whole of each hatch opening was then covered with the usual canvas tarpaulin.

Application of the Fumigant. The crew was evacuated from the vessel during the fumigation and subsequent aeration period.

The dose of 2 pounds of methyl bromide per thousand cubic feet, applied from 1-pound cans, was introduced through small openings in the

sealed hatch covers by means of "Saran" plastic tubing. The temperatures prevailing while the work was being done lay between 75° and 80° F. in the commodity and 72° to 80° in the free air space.

Two circulating fans were placed on top of the cargo, and were run for 30 minutes after the application of the fumigant. The exposure period was 24 hours, following which 12 hours were allowed for ventilation before any persons were allowed to work in the holds or return to the crew's quarters or engine room.

Results. Following the fumigation and also at the time of unloading of the cargo at Hamilton, Ontario, representative samples from all parts of the load were sifted and large numbers of dead insects recovered. In no case were any living stages of either of the two species, *T. castaneum* Herbst. and *O. surinamensis* L. found in any part of the ship or cargo.

Following the fumigation of one of the vessels, a bad odour was detected in the engine-room and cargo space, but this did not contaminate the peanuts in any way. The smell, which was dissipated within a few hours, was attributed to the reaction of the methyl bromide with the bilge water, which doubtless contained a large quantity of grease and oil.

FUMIGATION IN A GREAT LAKES FREIGHTER

In August 1946, a cargo of 2100 tons of Nigerian peanuts was moved from Montreal, Quebec, to Hamilton, Ontario, by a lake freighter SS. "Battleford" of the Canada Steamship Lines. This cargo was also infested with the red flour beetle *T. castaneum* Herbst. and the saw-toothed grain beetle *O. surinamensis* L., and arrangements were made to fumigate the cargo on board this vessel after she was fully loaded.

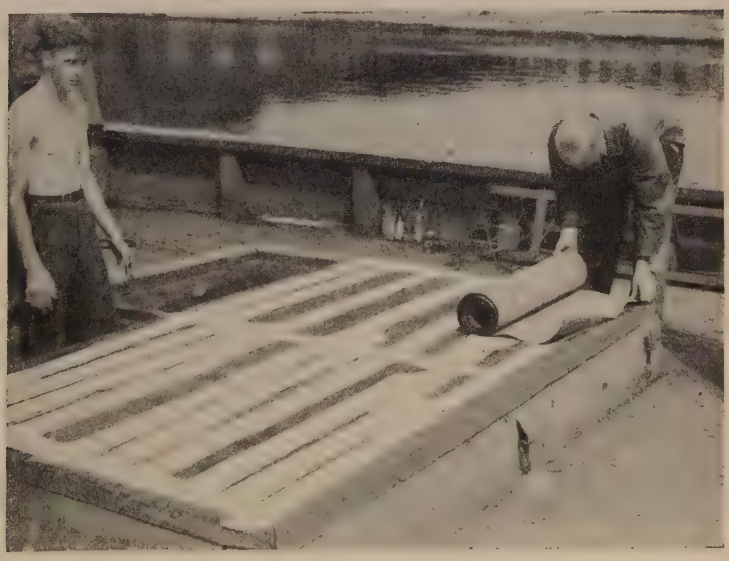

FIGURE 4. Sealing hatch cover of wooden schooner

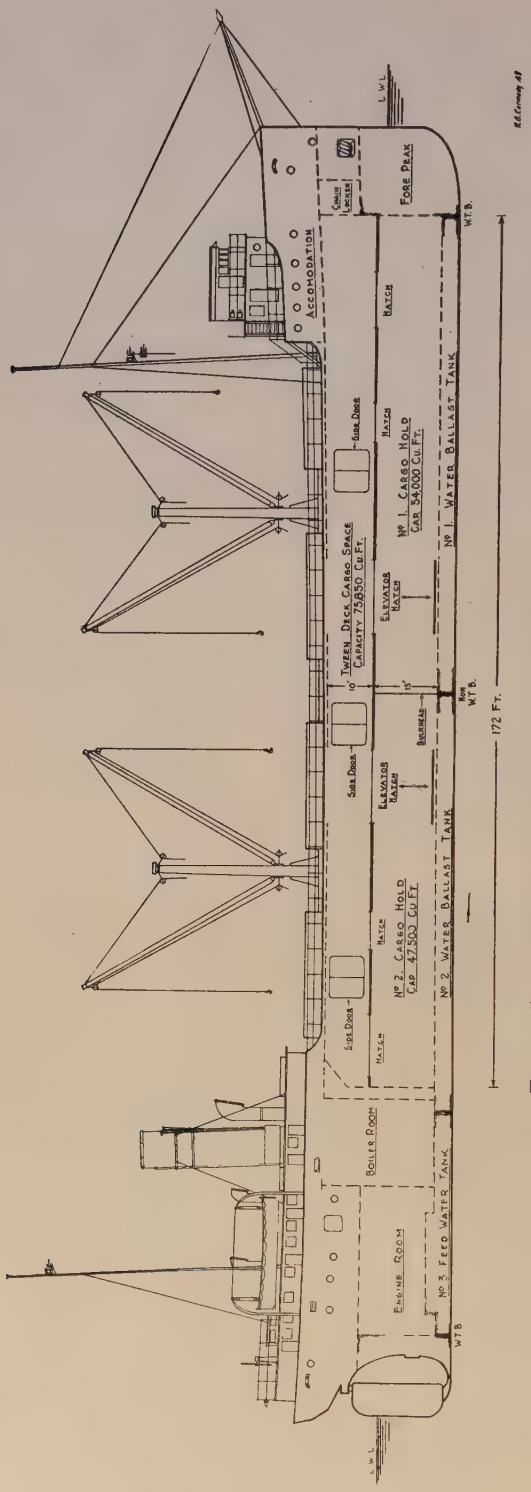

FIGURE 5. Longitudinal plan of package freighter SS. "Battleford".

Description of Vessel. The steamer, a longitudinal plan of which is shown in Figure 5, was a Great Lakes cargo vessel of the type known as a "package freighter", so-called because of the special type of construction employed, with side doors and elevators to move goods from the 'tween decks to the lower hold, which thus enabled packages to be moved easily on "pallet" boards with the aid of special lift trucks. These side doors were of great assistance during the aeration process, as will be demonstrated more fully later on.

The displacement weight of this vessel when empty was 2650 tons, while the hull had dimensions of: length, 248 feet; breadth, 43 feet; and depth, 25 feet.

Description of Cargo Space. The cargo space in the 'tween decks had no bulkheads and ran the whole length of the cargo deck, with dimensions of approximately 172 × 10 × 42 feet, giving a gross cubic capacity according to the steamship plans of 75,850 cubic feet.

The lower cargo space with a depth of 15 feet was divided by a steel bulkhead into numbers 1 and 2 cargo holds, with gross capacities of 54,000 and 47,500 cubic feet, respectively. The total cargo space in the ship was thus 177,410 cubic feet. Access to the lower holds was obtained by means of the two elevators or through the lower cargo hatches, as shown in Figure 6.

Method of Loading Cargo. The bags of peanuts, weighing approximately 200 pounds each, were placed 12 at a time on the special "pallet" boards used for handling cargo on the package freighters. The construction and use of the pallet boards can be clearly seen in Figures 7 and 8. Each "board" had dimensions of 66 × 46 × 4 inches and consisted essentially of two surfaces of slats separated by 2 × 4 inch boards, so that

FIGURE 7. Pallet boards holding peanuts conveyed into steamship hold.

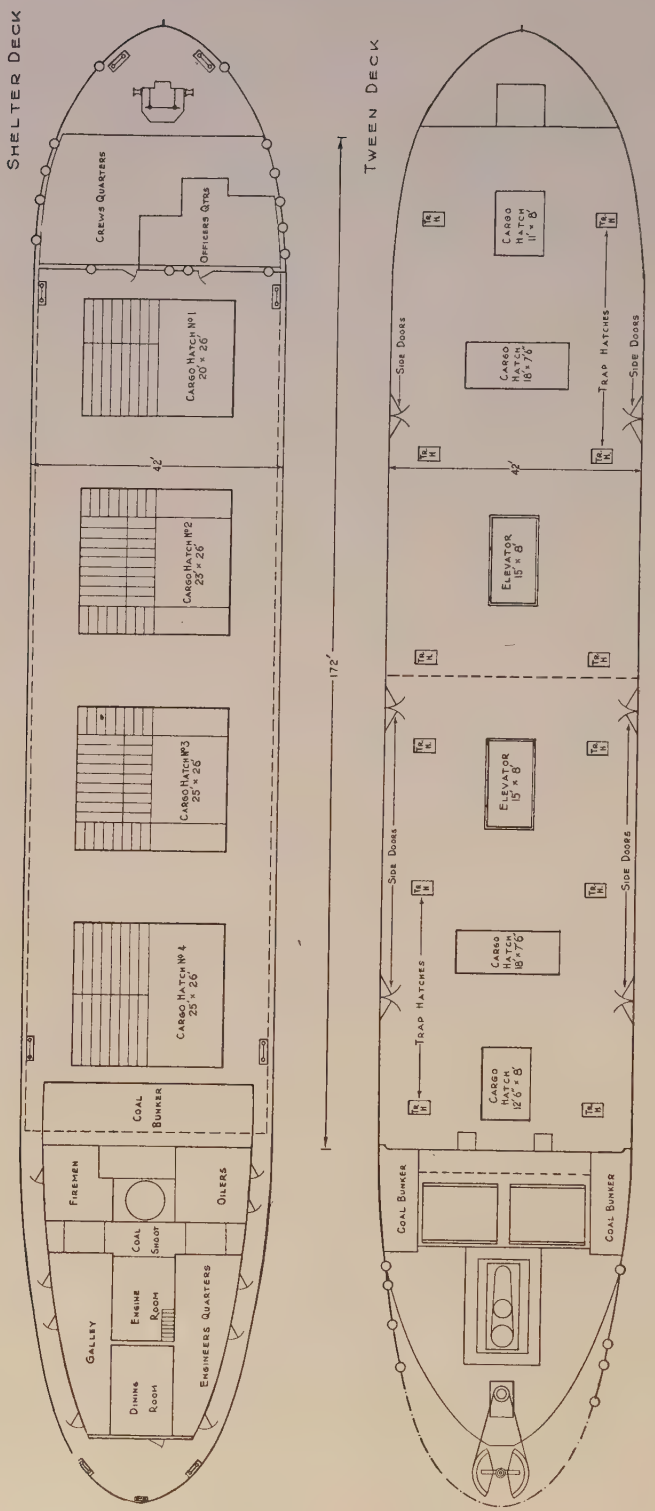

FIGURE 6. Deck plans of package freighter SS. "Battleford".

FIGURE 8. 'Tween decks of cargo space in package freighter, showing method of loading peanut bags on pallet boards; also boards over hatch leading to lower hold.

the prongs of the lift trucks, illustrated in Figure 7, could be inserted between to raise each load of bags, and convey it into the ship through the side doors, for fitting into place in the cargo.

The pallet boards with their bags were loaded three deep in the lower hold, giving a total depth of 12 bags, while in the 'tween deck space the pile was only two boards high, with a depth of 8 bags.

The use of these boards, it is believed, may have greatly facilitated the circulation and penetration of the fumigant throughout the cargo, and aided the subsequent ventilation.

On completion of the loading of the lower holds it was necessary to place the wooden boards across the hatches (see Figure 6) in order for cargo to be loaded in this space. The boards were not closely fitted, subsequently allowing for diffusion upwards of the gas concentrations in the lower holds, an arrangement which doubtless had considerable bearing on the subsequent ventilation of the gas from the lower cargo space.

The elevator platforms were also left at the bottom of the lower holds, but in this instance no boards were placed across the elevator hatch. It was possible to arrange for four vertical shafts of about two feet diameter to run all the way from the top hatches into the lower holds, which also aided in the ventilation of the fumigant following the completion of exposure.

In order to get the complete cargo on board the vessel, it was also necessary to heap some bags on top of the piles by loading them through the deck hatches.

FIGURE 9. Sealing hatch cover boards with paste and brown paper.

Sealing Technique. The sealing method was similar to that employed with the schooners, and is illustrated in Figures 9 and 10. It was not considered necessary to apply any external sealing to the side doors on the hull, as those were of water-tight construction and provided with rubber gaskets.

The only other places where sealing was thought to be necessary were the two small holes drilled through the bulkheads at each end of the 'tween decks to permit passage of the wires actuating the bridge-to-engine-room telegraph system. These were sealed with Kraftex paper and flour paste.

Application of Fumigant. The temperatures in the free air space of the boat were, at the time of fumigation 71° F., and at time of aeration 64° F. The temperatures in the peanuts ranged from 70° to 75° F.

For the cargo space of 177,410 cubic feet, 350 pounds of methyl bromide were used, approximately at the rate of 2 pounds per thousand cubic feet. The gas was applied through 3/16 of an inch inside diameter copper tubing from 50- and 100-pound cylinders placed on the top deck. Each lower hold received the contents of one 100-pound cylinder, and three 50-pound cylinders were used for the 'tween deck space.

The copper lines leading from the 100-pound cylinders to each of the lower holds were divided, after entering the lower hold, by means of a "T" into two lines leading in opposite directions, each with three final outlets. In the 'tween decks, the tubing from each cylinder leading to the ends of the space had three terminal outlets, while the line from the middle cylinder was divided by a "T" into two lines each with three terminal outlets.

Four electric fans with 10-inch blades were placed on top of the load, two in each of the lower holds. Following the application of the fumigant, these were operated for 30 minutes to aid circulation in the lower holds.

The exposure period, following the introduction of the gas, was 24 hours.

Leakage of the Fumigant. During the fumigation, tests with the Halide Leak Detector were made from time to time in the coal bunkers, engine-room space, accommodation, around the side doors, and all parts of the ship not under exposure to the gas. No indication of methyl bromide was found in any of these places and the only positive reaction was a slight one near the holes for the engine-room telegraph wires, mentioned above, which had apparently not been rendered completely gas-tight. These results indicated that to all intents and purposes the cargo space in these freighters can be considered as a gas-tight unit.

Ventilation. The outdoor temperatures prevailing during the entire period from the fumigation in Montreal on August 24, to the completion of unloading at Hamilton on August 31, ranged from a minimum of 46° F. on August 30, to a maximum of 76° F. on August 28. The commodity temperature remained at 70° to 75° F. during all this period.

At the end of the treatment period of 24 hours, the boards at each side of the hatch covers were quickly lifted up by the crew of fumigators, wearing gas-masks. Following this, the Halide Leak Detector showed a very strong blue flame in the vicinity of the boat. At intervals of thirty minutes, more boards were removed until after 8 hours all the hatches were completely uncovered. Eight hours after the initial opening the fumigators entered the 'tween decks and opened all six side doors from the inside. Twelve hours after opening, the cargo in the 'tween decks was inspected for insect survival and at the same time, there was no reaction on the Halide Leak Detector anywhere among the bags in this space. By climbing over the

FIGURE 10. Placing tar paper over boards for sealing hatch covers in package freighter.

bags a short distance into the lower hold, reactions were obtained indicating concentrations of methyl bromide of 50-100 parts per million. It was therefore considered safe to allow the crew to reoccupy the boat. Two hours were required to raise steam and the vessel sailed from the dock at 2.00 p.m.

Observations were continued on the ventilation of the fumigant during the voyage from Montreal to Hamilton, which lasted 64 hours. Soon after leaving the dock, showers of rain necessitated the replacing of the hatch covers and tarpaulins except for one board at each side. The side doors were not closed. At 5.00 p.m., three hours after sailing, and following a voyage through the entire length of the Lachine Canal, concentrations of 50-100 parts per million of methyl bromide were detected in the cargo space near the hatch covers. These concentrations continued to be detected during the following day, during which time the ship was moving mainly through canals and locks. Owing to the fact that the boat was soon due to leave the canal for the open stretches of the St. Lawrence River and the voyage across Lake Ontario, the side doors were closed during the evening, 30 hours after sailing, and at this time concentrations of 50-100 parts per million were detected between the bags and in the lower holds. As the open space near the doors was apparently clear of gas, it was considered safe for the crew to enter and close the doors for the short length of time required for this operation.

The following day, the vessel steamed at nine knots across the open water of Lake Ontario, in the face of a moderate west wind, and although the side doors were closed and only the side boards of each hatch cover were open, by evening (54 hours after sailing and 68 hours after opening up following the fumigation), it was impossible to find any trace of residual fumigant in any part of the cargo in the 'tween decks or lower hold accessible at that time. Next morning the steamer reached Hamilton and unloading of the cargo began immediately. During this process, which continued for three days, periodical tests were made in the holds where the men were unloading. At no time was any positive reaction for methyl bromide obtained, either in the free air space or among the bags. The unloading, therefore, was conducted under conditions of safety to all concerned.

In reviewing the above account of the ventilation process, it is believed that the partial replacement of the hatch covers after the boat sailed may have retarded the aeration, while closing the doors at the end of 30 hours hampered this process still further. The movement across the open lake, together with the fresh breeze, apparently brought about complete and final ventilation during the third day.

From the evidence presented by the absence of detectable concentrations in the 'tween deck cargo, immediately prior to departure of the boat, while side doors and all hatch covers were fully open, it is concluded that the vapours of 50-100 parts per million subsequently detected represented diffusion upwards from the lower hold, possibly accelerated by the vibration of the ship.

During the entire voyage no positive indications for methyl bromide were detected in the crew quarters, officers' cabins, bunkers, engine-room or, indeed, any important space outside of that occupied by the cargo.

However, slight traces were found where the telegraph wires came through from the 'tween decks into lockers in the forepeak of the ship.

Toxicity Results. Intensive sampling and inspection of both the outside and the contents of bags from all parts of the hold during the unloading of the cargo revealed the presence of no living survivors of either of the two species present, *T. castaneum*, Herbst. and *O. surinamensis* L. Subsequent examination of the samples after intervals of one and two months failed to show any development of other stages, thus indicating that apparently a complete mortality of the two species had been obtained.

DISCUSSION

The fumigations with methyl bromide described in this article have indicated that treatments can be carried out with prospects of success in a variety of types of barges and ships, providing that a fair degree of gas-tightness can be provided both by the construction of the vessel itself and by the use of efficient sealing methods.

This work has added somewhat to the information available on the effective penetration of methyl bromide through piles of bags. In the case of the diesel schooners with wooden hulls it would seem that most of the fumigant had to travel down through the pile itself, as the bags at the sides were pressed against the hull, a situation unlike that found in warehouses, where the gas can diffuse in from three or four sides. It may therefore be considered that the methyl bromide penetrated downward through solid piles of peanut bags for a distance of 10 to 12 feet.

In the case of the larger package freighter it appears that no definite conclusions can be drawn regarding depth of penetration as the load was consistently broken up by the air space provided by the pallet boards. Indeed, the presence of these boards may have been the principal factor ensuring the success of this fumigation.

Particular attention was paid to the ventilation of the fumigant from the loads following treatment. In the case of the large steamer, aeration was facilitated by the presence of the side doors in the hull and the use of the pallet boards. Even with these aids, it appeared that ventilation from the lower holds was slow, and atmospheres safe for men to work in were reached only 68 hours after aeration began.

From this work the conclusion is drawn that any further extension of methyl bromide fumigation to the holds of larger ships of the ocean-going type would be attended with considerable difficulty, especially in connection with the aeration subsequent to fumigation. Such work might be undertaken if modifications of the present methods of loading were brought about, including the provision of suitable ducts for drawing out the high gas concentrations from the deeper recesses of the holds.

SUMMARY

1. The fumigation with methyl bromide of peanuts imported in bags from tropical countries has been extended to river barges, wooden schooners, and Great Lakes freighters.

2. Barges of the type used on the Mississippi River were used in this work, but a large amount of sealing was required to make them sufficiently gas-tight.

3. The success of the fumigation on the lake freighter was apparently mainly due to the fact that the bags were loaded on pallet boards, thus ensuring considerable air space throughout the load.

4. For temperatures above 60° F., a dose of 2 pounds of methyl bromide per thousand cubic feet is at present recommended for an exposure period of 24 hours, with the provision of circulating fans to ensure the even distribution of the fumigant. This treatment was found satisfactory to control all stages of five species of insects commonly found in imported peanuts.

5. In the barges and schooners ventilation of the methyl bromide from the cargo spaces, as tested by the Halide Leak Detector, was fairly rapid owing to the limited depths of the piles of bags and the comparatively large area of the hatch covers as compared with the cargo space.

6. Final aeration of the fumigant from the lower hold of a Great Lakes package freighter took at least 68 hours to complete.

ACKNOWLEDGMENTS

Acknowledgment is due to Dr. P. N. Annand, Chief, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, who kindly provided the services of entomologists and plant quarantine inspectors to aid in the supervision of the barge fumigations. These officers included Mr. J. C. Frankenfeld of the Stored Product Insect Investigations Laboratory at Manhattan, Kansas, and Messrs. B. H. Petfield, C. P. Keeler, and W. E. Colvin, all of the above Bureau. The author is indebted to Dr. H. E. Gray of the Division of Entomology, Science Service, Ottawa, who in the absence of the writer, visited Pittsburgh, Pennsylvania to make arrangements for, and to supervise the fumigation in the Mississippi barges. The fumigation of the barges was conducted by the Commonwealth Sanitation Co., Pittsburgh, Pa. and during the first fumigation, Messrs. J. C. Dawson, R. Borg and T. Riedeburg, representatives of the Dow Chemical Co., Midland, Mich., were present to give technical assistance, especially in connection with the sealing methods.

The fumigations of the diesel schooners and the package freighter were conducted by Pestroy Ltd., of Montreal, under the direction of Mr. G. E. Worth, Manager. In the case of these fumigations, thanks are due to Mr. W. St. G. Ryan, District Inspector, Division of Plant Protection, Montreal, P.Q., for valuable assistance in supervising this work and inspecting the cargoes after treatment. Mr. G. L. Giasson, Inspector of the Montreal staff of this Division, was very helpful in gathering technical data both before and after the fumigations. Mr. A. Desjardins of the staff of this Laboratory assisted in the inspection of the cargoes during unloading at Hamilton.

In connection with the package freighter fumigation, thanks are due to the following officials of the Canada Steamship Lines for valued co-operation and advice: Captain N. J. Reoch, Operating Manager; Mr. L. J. Stock, Superintendent of Operations; Mr. W. J. Hughes, Terminal Superintendent, Montreal, P.Q.; Captain A. J. Galloway, Master of the SS. "Battleford".

The illustration of the plans of the freighter in Figures 5 and 6 were kindly prepared by Mr. H. G. Carmody of the Toronto Office of this Division, while Mr. R. Delisle of this Laboratory, helped in the preparation of the manuscript.

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The Veterinary journal: October, 1943.
The Veterinary record: Feb. 24, and March 30, 1940.
The Veterinary record: March 15, 1941.
The Veterinary record: Index, January-December, 1942.

It would be appreciated if anyone having extra copies of the above numbers would supply them to the library in order that the volumes to which they relate may be bound.

BOOK REVIEW

LAND CLASSIFICATION IN THE WEST MIDLAND REGION. West Midland Group on Post-War Reconstruction and Planning, Bournville, Birmingham, 30, England. Distributed by Faber & Faber, 24 Russell Square, London, W.C. 1. 12/6.

"Land Classification in the West Midland Region" is published by the West Midland Group on Post-War Reconstruction and Planning. This organization was brought into existence in 1942 as a result of the publication of the Barlow Report, which emphasized the need for pre-planning surveys. It is closely linked with the University of Birmingham, the Vice-Chancellor of wh.ch, Dr. Raymond Priestley, is Chairman of the Group. The Bournville Village Trust provides the finances, office accommodation and staff. The Group felt that the best service it could render would be by discovering, assembling and making available in published form the sort of information which is the prerequisite of good planning.

Even before the publication of the Barlow and Scott Reports, there was plenty of evidence to show that the urbanization of the English countryside was proceeding at a rate which was threatening to obliterate forever not just the pleasures of the countryside, but the whole heritage of culture and beauty associated with it. The war brought the realization of the enormous damage which urban spread had done to the agricultural industry and to the principal source of the nation's food.

Planning must have regard to the proper use of land and, as a general rule, the claims of agriculture for the use of the best agricultural land must be regarded as paramount. With this in mind, the Group considered that it was of the first importance to establish the location of the types of agricultural land in the West Midland region. A detailed scheme of land classification, based upon a field-by-field soil examination of the factors involved, would take probably 30-40 years to complete for the whole country. In order, however, that all those now concerned in town and country planning should have the essential facts available to them, it was decided by the conference of experts called by the Group to go ahead with the generalised classification scheme of the present work.

The basis of the scheme of land classification has been worked out by a Committee representing many varieties of experience and interest. The principle formulated by the Group of judging fertility by factors of site and soil, *i.e.*, of potential or inherent fertility rather than by its productivity under existing conditions, was new, and appears now to be fairly widely adopted in official surveys.

The scheme, as outlined, has been used for providing the land classification data included in the recently-published planning survey of Herefordshire, "English County", and will again be employed in another forthcoming publication of the Group relating to the area of the Birmingham-Wolverhampton-Black Country Conurbation.

—*By the authors.*

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